

## IN THE CLAIMS

1. (Previously presented) A method of obtaining new astaxanthin overproducing strains of *Xanthophyllomyces dendrorhous* (*X. dendrorhous*) consisting in inducing mutation of a parent strain of *X. dendrorhous* by incubating said parent strain under mutagenic conditions and by selecting the mutants obtained thereof, characterised because a first selection of astaxanthin overproducing mutants is achieved by growing the mutants on a solid medium containing either inhibitors of the synthesis of steroids or compounds that alter the redox potential of the cell and then astaxanthin overproducing mutant strains were selected subsequently as a function of their yield on solid medium measured as:

- i. deeper red coloration than the parent strain
- ii. production of astaxanthin in darkness
- iii. production of astaxanthin at temperatures near 20°C
- iv. production of astaxanthin using sucrose as carbon

source.

2. (Previously presented) A method according to claim 2, characterised by inducing mutation of the parent strain by incubating said strain in an appropriate culture medium containing a mutagenic agent selected among ethylmethanesulfonate (EMS) and N-methyl-N'-nitrosoguanidine (NTG) or irradiating said culture medium containing the parent strain of *X. dendrorhous* with ultraviolet arrays (UVA).

3. (Currently Amended) A method according to ~~any of the claims~~ claim 1 [[or 2]], in which the parent strain of *X. dendrorhous* was VKPM Y-2976.

4. (Currently Amended) A method according to ~~any of the claims~~ claim 1 [[-3]],

characterised by selecting the mutants by growing them in solid medium containing as inhibitor of the steroid synthesis a compound selected among  $\beta$ -ionone, imidazole, diethylamine, 2-methylimidazole, nystatin and diphenylamine or, as compound that alter the redox potential of the cell, a compound selected among duroquinone or hydrogen peroxide.

5. (Currently Amended) A method according to ~~any of the claims~~ claim 1 [[-4]], characterised by selecting the mutants by growing them in solid medium at 24°C.

6. (Currently Amended) Astaxanthin overproducing mutants obtainable by the method of claim[[s]] 1 [[-5]], characterised by possessing extrachromosomal elements consisting in linear double strand DNA plasmids and capable of producing at least 4000 ppm of astaxanthin after 6-7 days by flask fermentation.

7. (Previously Presented) Astaxanthin overproducing mutants according to claim 6, characterised by producing at least 5000 ppm of astaxanthin after 7-9 days in industrial fermentation.

8. (Currently Amended) Process for producing astaxanthin characterised in culturing in a suitable medium at appropriate growth conditions the mutants of claim[[s]] 6 [[or 7]] or derivatives thereof having the same extra chromosomal elements and having the same level of astaxanthin production.

9. (Previously Presented) A process of fermentation according to claim 8, characterized in that duroquinone is added during the fermentation process.

10. (Previously Presented) A process of fermentation according to claim 9, characterized

in that duroquinone is added at a concentration of 25-50  $\mu$ M.

11. (Previously Presented) A process of fermentation according to claim 8, characterized in that retinal is added during the fermentation process.

12. (Previously Presented) A process of fermentation according to claim 11, characterized in that retinal is added at a concentration of 35  $\mu$ M.

13. (Previously Presented) A process of fermentation according to claim 6, characterized in that trisporic acids are added during the fermentation process.

14. (Previously Presented) A process of fermentation according to claim 13, characterized in that the trisporic acids are added at a concentration of 50-100  $\mu$ g/ml.

15. (Previously Presented) A process of fermentation according to claim 8, characterized in that glutamate is added during the fermentation process.

16. (Previously Presented) A process of fermentation according to claim 15, characterized in that glutamate is added at a concentration of 5.5 mg/ml.

17. (Previously Presented) A process of fermentation according to claim 8, characterized in that medium 5 described in Table I of the description is used for the fermentation process.

18. (Currently Amended) A process of fermentation according to ~~any of the claims~~ claim 8 [[-17]], characterized in that the fermentation medium is illuminated during the fermentation process.

19. (Previously Presented) A process of fermentation according to claim 18, characterized in that the source of illumination used is white light.

20. (Previously Presented) A process of fermentation according to claim 18, characterized in that the source of illumination used is ultraviolet light.

21. (Currently Amended) A process of fermentation according to ~~any of the claims~~ claim 18 ~~[[20]]~~, characterized in that illumination is carried out from the start to the end of fermentation, preferably from 40 to 200 hours.

22. (Previously Presented) A process of fermentation according to claim 21, characterized in that cycles of 6 hours of illumination / darkness are used.

23. (Currently Amended) A process of fermentation according to ~~any of the claims~~ 8 ~~[[22]]~~, characterized in that:

- (a) Inocula of *X. dendrorhous* are seeded.
- (b) The inocula of *X. dendrorhous* are cultivated for 48 hours at 20°C.
- (c) Phases of primary culture of *X. dendrorhous* are seeded with about 0.41% (v/v) of the inoculum phase.
- (d) The primary phases of *X. dendrorhous* are cultivated for 48-54 hours at 17-20°C.
- (e) Each fermenter is seeded with 20% (v/v) of the primary phases of *X. dendrorhous*.
- (f) The fermentation is incubated at 18-20°C for 60-72 hours and then at 17°C for 5-7 days.

24. (Currently Amended) Biomass of *X. dendrorhous* with nutrient and pigmenting value, obtainable by the fermentation process described in claim[[s]] 8 [[to 23]], for use in food for humans and animals.

25. (Previously Presented) Biomass according to claim 24, characterized in that it contains:

- a) A concentration of at least 5000 µg/g of astaxanthin;
- b) A concentration of at least 7400 µg/g of total  
carotenoids;
- c) A concentration of at least 15% of proteins and
- d) A concentration of at least 15% of carbohydrates.

26. (Currently Amended) Compounds for animal food that consist of or contain the biomass of claim[[s]] 24 [[and 25]].

27. (Currently Amended) Compounds for human food that consist of or contain the biomass of claim[[s]] 24 [[and 25]].